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FILE 'HOME' ENTERED AT 17:51:58 ON 30 MAY 2001

=> file medline biosis biotechno

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.45	0.45

FILE 'MEDLINE' ENTERED AT 17:53:36 ON 30 MAY 2001

FILE 'BIOSIS' ENTERED AT 17:53:36 ON 30 MAY 2001

FILE 'BIOTECHNO' ENTERED AT 17:53:36 ON 30 MAY 2001

=> s G-protein or 7tm or (seven transmembrane) or (seven membrane)

L1 56521 G-PROTEIN OR 7TM OR (SEVEN TRANSMEMBRANE) OR (SEVEN MEMBRANE)

=> s l1 and ((cytoplasmic loop?) or (intracellular loop?))

L2 1216 L1 AND ((CYTOPLASMIC LOOP?) OR (INTRACELLULAR LOOP?))

=> s l2 and chimer? or fusion or fused

L3 219027 L2 AND CHIMER? OR FUSION OR FUSED

=> s l2 and (chimer? or fusion or fused)

L4 232 L2 AND (CHIMER? OR FUSION OR FUSED)

=> s l4 and rhodopsin

L5 19 L4 AND RHODOPSIN

=> dup rem l5

PROCESSING COMPLETED FOR L5
L6 8 DUP REM L5 (11 DUPLICATES REMOVED)

=> d ibib abs 1-8

L6 ANSWER 1 OF 8 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2001106004 MEDLINE
DOCUMENT NUMBER: 20564305 PubMed ID: 10988291
TITLE: Functionally discrete mimics of light-activated **rhodopsin** identified through expression of soluble cytoplasmic domains.
AUTHOR: Abdulaev N G; Ngo T; Chen R; Lu Z; Ridge K D
CORPORATE SOURCE: Center for Advanced Research in Biotechnology, National Institute of Standards and Technology and the University of Maryland Biotechnology Institute, Rockville, Maryland 20850, USA.
CONTRACT NUMBER: EY11112 (NEI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Dec 15) 275 (50) 39354-63.
Journal code: HIV. ISSN: 0021-9258.

PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200102
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered PubMed: 20010108
Entered Medline: 20010208

AB Numerous studies on the seven-helix receptor **rhodopsin** have implicated the **cytoplasmic loops** and carboxyl-terminal region in the binding and activation of proteins involved in visual transduction and desensitization. In our continuing studies on **rhodopsin** folding, assembly, and structure, we have attempted to reconstruct the interacting surface(s) for these proteins by inserting fragments corresponding to the **cytoplasmic loops** and/or the carboxyl-terminal tail of bovine opsin either singly, or in combination, onto a surface loop in thioredoxin. The purpose of the thioredoxin **fusion** is to provide a soluble scaffold for the cytoplasmic fragments thereby allowing them sufficient conformational freedom to fold to a structure that mimics the protein-binding sites on light-activated **rhodopsin**. All of the **fusion** proteins are expressed to relatively high levels in Escherichia coli and can be purified using a two- or three-step chromatography procedure. Biochemical studies show that some of the **fusion** proteins effectively mimic the activated conformation(s) of **rhodopsin** in stimulating **G-protein** or competing with the light-activated **rhodopsin/G-protein** interaction, in supporting phosphorylation of the carboxyl-terminal opsin fragment by **rhodopsin** kinase, and/or phosphopeptide-stimulated arrestin binding. These results suggest that specific segments of the cytoplasmic surface of **rhodopsin** can adopt functionally discrete conformations in the absence of the connecting transmembrane helices and retinal chromophore.

L6 ANSWER 2 OF 8 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2001048370 MEDLINE
DOCUMENT NUMBER: 20507869 PubMed ID: 10930404
TITLE: Distinct roles of the second and third **cytoplasmic loops** of bovine **rhodopsin** in **G protein** activation.
AUTHOR: Yamashita T; Terakita A; Shichida Y
CORPORATE SOURCE: Department of Biophysics, Graduate School of Science, Kyoto University, Japan.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Nov 3) 275 (44) 34272-9.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200012
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered PubMed: 20001120
Entered Medline: 20001214

AB In contrast to the extensive studies of light-induced conformational changes in **rhodopsin**, the cytoplasmic architecture of **rhodopsin** related to the **G protein** activation and the selective recognition of **G protein** subtype is still unclear. Here, we prepared a set of bovine **rhodopsin** mutants whose **cytoplasmic loops** were replaced by those of other ligand-binding receptors, and we compared their ability for **G protein** activation in order to obtain a clue to the roles of the second and third **cytoplasmic loops** of **rhodopsin**. The mutants bearing the third loop of four other G(o)-coupled receptors belonging to the **rhodopsin** superfamily showed significant G(o) activation, indicating that the third loop of

rhodopsin possibly has a putative site(s) related to the interaction of **G protein** and that it is simply exchangeable with those of other G(o)-coupled receptors. The mutants bearing the second loop of other receptors, however, had little ability for **G protein** activation, suggesting that the second loop of **rhodopsin** contains a specific region essential for **rhodopsin** to be a **G protein**-activating form. Systematic **chimeric** and point mutational studies indicate that three amino acids (Glu(134), Val(138), and Cys(140)) in the N-terminal region of the second loop of **rhodopsin** are crucial for efficient **G protein** activation. These results suggest that the second and third **cytoplasmic loops** of bovine **rhodopsin** have distinct roles in **G protein** activation and subtype specificity.

L6 ANSWER 3 OF 8 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2000102706 MEDLINE
DOCUMENT NUMBER: 20102706 PubMed ID: 10636894
TITLE: The amino terminus of the fourth **cytoplasmic loop** of **rhodopsin** modulates **rhodopsin**-transducin interaction.
AUTHOR: Marin E P; Krishna A G; Zvyaga T A; Isele J; Siebert F; Sakmar T P
CORPORATE SOURCE: Laboratory of Molecular Biology and Biochemistry, The Rockefeller University, New York, New York 10021, USA.
CONTRACT NUMBER: EY07138 (NEI)
GM07739 (NIGMS)
GM07982 (NIGMS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Jan 21) 275 (3) 1930-6.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200002
ENTRY DATE: Entered STN: 20000309
Last Updated on STN: 20000309
Entered Medline: 20000224

AB **Rhodopsin** is a **seven-transmembrane** helix receptor that binds and catalytically activates the heterotrimeric **G protein** transducin (G(t)). This interaction involves the cytoplasmic surface of **rhodopsin**, which comprises four putative loops and the carboxyl-terminal tail. The fourth loop connects the carboxyl end of transmembrane helix 7 with Cys(322) and Cys(323), which are both modified by membrane-inserted palmitoyl groups. Published data on the roles of the fourth loop in the binding and activation of G(t) are contradictory. Here, we attempt to reconcile these conflicts and define a role for the fourth loop in **rhodopsin**-G(t) interactions. Fluorescence experiments demonstrated that a synthetic peptide corresponding to the fourth loop of **rhodopsin** inhibited the activation of G(t) by **rhodopsin** and interacted directly with the alpha subunit of G(t). A series of **rhodopsin** mutants was prepared in which portions of the fourth loop were replaced with analogous sequences from the beta(2)-adrenergic receptor or the m1 muscarinic receptor. **Chimeric** receptors in which residues 310-312 were replaced could not efficiently activate G(t). The defect in G(t) interaction in the fourth loop mutants was not affected by preventing palmitoylation of Cys(322) and Cys(323). We suggest that the amino terminus of the fourth loop interacts directly with G(t), particularly with Galpha(t), and with other regions of the intracellular surface of **rhodopsin** to support G(t) binding.

L6 ANSWER 4 OF 8 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 1999315899 MEDLINE
DOCUMENT NUMBER: 99315899 PubMed ID: 10383462
TITLE: Two **cytoplasmic loops** of the glucagon

eptor are required to elevate P or intracellular calcium.

AUTHOR: Cypess A M; Unson C G; Wu C R; Sakmar T P
CORPORATE SOURCE: The Rockefeller University, New York, New York 10021, USA.
CONTRACT NUMBER: CA09673 (NCI)
DK24039 (NIDDK)
DK54718 (NIDDK)
+
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Jul 2) 274 (27) 19455-64.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U14012
ENTRY MONTH: 199907
ENTRY DATE: Entered STN: 19990806
Last Updated on STN: 19990806
Entered Medline: 19990727

AB The glucagon receptor is a member of a distinct class of **G protein**-coupled receptors (GPCRs) sharing little amino acid sequence homology with the larger **rhodopsin**-like GPCR family. To identify the components of the glucagon receptor necessary for **G -protein** coupling, we replaced sequentially all or part of each **intracellular loop** (i1, i2, and i3) and the C-terminal tail of the glucagon receptor with the 11 amino acids comprising the first **intracellular loop** of the D4 dopamine receptor. When expressed in transiently transfected COS-1 cells, the mutant receptors fell into two different groups with respect to hormone-mediated signaling.

The first group included the loop i1 mutants, which bound glucagon and signaled normally. The second group comprised the loop i2 and i3 **chimeras**, which caused no detectable adenylyl cyclase activation in COS-1 cells. However, when expressed in HEK 293T cells, the loop i2 or i3 **chimeras** caused very small glucagon-mediated increases in cAMP levels and intracellular calcium concentrations, with EC50 values nearly 100-fold higher than those measured for wild-type receptor. Replacement of both loops i2 and i3 simultaneously was required to completely abolish **G protein** signaling as measured by both cAMP accumulation and calcium flux assays. These results show that the i2 and i3 loops play a role in glucagon receptor signaling, consistent with recent models for the mechanism of activation of G proteins by **rhodopsin**-like GPCRs.

L6 ANSWER 5 OF 8 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1999:526182 BIOSIS
DOCUMENT NUMBER: PREV199900526182
TITLE: Bound conformations for ligands for **G-protein** coupled receptors.
AUTHOR(S): Marshall, Garland R. (1); Ragno, Rino (1); Makara, Gergely M. (1); Arimoto, Rieko (1); Kisselev, Oleg
CORPORATE SOURCE: (1) Center for Molecular Design, Department of Molecular Biology and Pharmacology, Washington University, Saint Louis, MO, 63110 USA
SOURCE: Letters in Peptide Science, (Sept., 1999) Vol. 6, No. 5-6, pp. 283-288.
ISSN: 0929-5666.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The conformation of the C-terminus of the alpha-subunit of transducin, the **G-protein** of vision, has been determined by transfer NOE when bound to activated (MII) **rhodopsin**. One hundred three new NOE constraints are apparent when light is shown on a mixture of

rhodopsin bila and the undecapeptide. Analo[redacted]f the alpha-peptide with covalent constraints were designed restricting the bound conformation; they stabilize MII thus supporting the deduced structure. The NMR structure of a complex of the **intracellular loops** of **rhodopsin** facilitates docking of the alpha-peptide and also shows proximity of residues known by mutational analysis to interact to generate the activated **rhodopsin** -transducin interface. This constrains the location of transmembrane helices in the structure of activated **rhodopsin**. Methods for the prediction of affinity have been used to estimate the relative binding constants of peptide analogs with the loop complex and show strong correlation with experimental data. Various models of the **rhodopsin**-transmembrane helical segments have been computationally **fused** with distance geometry to determine the overall model which best fits the experimental data on the **rhodopsin**-transducin interface.

L6 ANSWER 6 OF 8 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 97400575 MEDLINE
DOCUMENT NUMBER: 97400575 PubMed ID: 9252410
TITLE: Stimulus-dependent phosphorylation of **G-protein**-coupled receptors by casein kinase 1alpha.
AUTHOR: Tobin A B; Totty N F; Sterlin A E; Nahorski S R
CORPORATE SOURCE: Department of Cell Physiology and Pharmacology, University of Leicester, University Road, Leicester LE1 9HN, United Kingdom.. TBA@le.ac.uk
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 Aug 15) 272 (33) 20844-9.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199709
ENTRY DATE: Entered STN: 19970916
Last Updated on STN: 20000303
Entered Medline: 19970904

AB We have previously demonstrated that the phospholipase C-coupled m3-muscarinic receptor is phosphorylated in an agonist-sensitive manner by a protein kinase of approximately 40 kDa purified from porcine cerebellum (Tobin, A. B., Keys, B., and Nahorski, S. R. (1996) J. Biol Chem. 271, 3907-3916). This kinase, called muscarinic receptor kinase (MRK), is distinct from second messenger-regulated protein kinases and from beta-adrenergic receptor kinase and other members of the **G-protein**-coupled receptor kinase family. In the present study we propose that MRK is casein kinase 1alpha (CK1alpha) based on the following evidence: 1) the amino acid sequence from two proteolytic peptide fragments derived from purified MRK corresponded exactly to sequences within CK1alpha. 2) Casein kinase activity co-eluted with MRK activity from the final two chromatography steps in the purification of porcine brain MRK. 3) Recombinant CK1alpha expressed in Sf9 cells is able to phosphorylate both casein and the bacterial **fusion** protein, Ex-m3, that contains a portion of the third **intracellular loop** of the m3-muscarinic receptor downstream of glutathione S-transferase. 4) Partially purified CK1alpha increased the level of muscarinic receptor phosphorylation in an agonist-sensitive manner when reconstituted with membranes from Chinese hamster ovary-m3 cells expressing the human recombinant m3-muscarinic receptor. 5) Partially-purified CK1alpha phosphorylated **rhodopsin**, contained in urea-treated bovine rod outer segment membranes, and the extent of phosphorylation was increased in the presence of light. These data demonstrate that the kinase previously called MRK is CK1alpha, and that CK1alpha offers an alternative protein kinase pathway from that of the **G-protein**-coupled receptor kinase family for the stimulus-dependent phosphorylation of the m3-muscarinic receptor, **rhodopsin**, and possibly other **G-protein** -coupled receptors.

L6 ANSWER 7 OF 8 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 97178816 MEDLINE
DOCUMENT NUMBER: 97178816 PubMed ID: 9063879
TITLE: Ca2+-dependent inhibition of **G protein**-coupled receptor kinase 2 by calmodulin.
AUTHOR: Haga K; Tsuga H; Haga T
CORPORATE SOURCE: Department of Biochemistry, Institute for Brain Research, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Japan.. haga@m.u-tokyo.ac.jp
SOURCE: BIOCHEMISTRY, (1997 Feb 11) 36 (6) 1315-21.
Journal code: A0G; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199703
ENTRY DATE: Entered STN: 19970327
Last Updated on STN: 19980206
Entered Medline: 19970317

AB Agonist- or light-dependent phosphorylation of muscarinic acetylcholine receptor m2 subtypes (m2 receptors) or **rhodopsin** by **G protein**-coupled receptor kinase 2 (GRK2) was found to be inhibited by calmodulin in a Ca2+-dependent manner. The phosphorylation was fully inhibited in the absence of **G protein** betagamma subunits and partially inhibited in the presence of betagamma subunits. The dose-response curve for stimulation by betagamma subunits of the m2 and **rhodopsin** phosphorylation was shifted to the higher concentration of betagamma subunits by addition of Ca2+-calmodulin. The phosphorylation by GRK2 of a glutathione S-transferase **fusion** protein containing a peptide corresponding to the central part of the third **intracellular loop** of m2 receptors (I3-GST) was not affected by Ca2+-calmodulin in the presence or absence of betagamma subunits, but the agonist-dependent stimulation of I3-GST phosphorylation by an I3-deleted m2 receptor mutant in the presence of betagamma subunits was suppressed by Ca2+-calmodulin. These results indicate that Ca2+-calmodulin does not directly interact with the catalytic site of GRK2 but inhibits the kinase activity of GRK2 by interfering with the activation of GRK2 by agonist-bound m2 receptors and **G protein** betagamma subunits. In agreement with the assumption that GRK2 activity is suppressed by the increase in intracellular Ca2+, the sequestration of m2 receptors expressed in Chinese hamster ovary cells was found to be attenuated by the treatment with a Ca2+ ionophore, A23187.

L6 ANSWER 8 OF 8 MEDLINE
ACCESSION NUMBER: 95047914 MEDLINE
DOCUMENT NUMBER: 95047914 PubMed ID: 7959413
TITLE: Regulation of **G protein**-coupled receptor kinase activity.
AUTHOR: Haga T; Haga K; Kameyama K; Nakata H
CORPORATE SOURCE: Institute for Brain Research, Faculty of Medicine, University of Tokyo, Japan.
SOURCE: NIPPON YAKURIGAKU ZASSHI. FOLIA PHARMACOLOGICA JAPONICA, (1994 Sep) 104 (3) 207-16. Ref: 50
Journal code: F2X; 0420550. ISSN: 0015-5691.
PUB. COUNTRY: Japan
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: Japanese
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199412
ENTRY DATE: Entered STN: 19950110
Last Updated on STN: 20000303
Entered Medline: 19941214

AB Recent progress on the activation of **G protein**-coupled receptor kinases is reviewed. beta-Adrenergic receptor kinase (beta ARK)

is activated by **protein** beta gamma -subunits, [redacted]ch interact with the carboxyl terminal portion of beta ARK. Muscarinic receptor m2-subtypes are phosphorylated by beta ARK1 in the central part of the third **intracellular loop** (I3). Phosphorylation of I3-GST **fusion** protein by beta ARK1 is synergistically stimulated by the beta gamma -subunits and mastoparan or a peptide corresponding to portions adjacent to the transmembrane segments of m2-receptors or by beta gamma -subunits and the agonist-bound I3-deleted m2 variant. These results indicate that agonist-bound receptors serve as both substrates and activators of beta ARK.

=> d his

(FILE 'HOME' ENTERED AT 17:51:58 ON 30 MAY 2001)

FILE 'MEDLINE, BIOSIS, BIOTECHNO' ENTERED AT 17:53:36 ON 30 MAY 2001
L1 56521 S G-PROTEIN OR 7TM OR (SEVEN TRANSMEMBRANE) OR (SEVEN MEMBRANE)
L2 1216 S L1 AND ((CYTOPLASMIC LOOP?) OR (INTRACELLULAR LOOP?))
L3 219027 S L2 AND CHIMER? OR FUSION OR FUSED
L4 232 S L2 AND (CHIMER? OR FUSION OR FUSED)
L5 19 S L4 AND RHODOPSIN
L6 8 DUP REM L5 (11 DUPLICATES REMOVED)

=> s l4 and py <1999

2 FILES SEARCHED...
L7 176 L4 AND PY <1999

=> dup rem l7

PROCESSING COMPLETED FOR L7
L8 79 DUP REM L7 (97 DUPLICATES REMOVED)

=> d ibib abs 1-79

L8 ANSWER 1 OF 79 MEDLINE
ACCESSION NUMBER: 1998434564 MEDLINE
DOCUMENT NUMBER: 98434564 PubMed ID: 9756892
TITLE: Molecular basis of V2 vasopressin receptor/Gs coupling selectivity.
AUTHOR: Erlenbach I; Wess J
CORPORATE SOURCE: Laboratory of Bioorganic Chemistry, NIDDK, National Institutes of Health, Bethesda, Maryland 20892, USA.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1998 Oct 9)** 273 (41) 26549-58.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199811
ENTRY DATE: Entered STN: 19990106
Last Updated on STN: 20000303
Entered Medline: 19981102

AB The molecular mechanisms governing the coupling selectivity of **G protein**-coupled receptors activated by peptide ligands are not well understood. To shed light on this issue, we have used the Gq/11-linked V1a and the Gs-coupled V2 vasopressin peptide receptors as model systems. To explore the structural basis underlying the ability of the V2 receptor to selectively recognize Gs, we systematically substituted distinct V2 receptor segments (or single amino acids) into the V1a receptor and studied whether the resulting hybrid receptors gained the ability to mediate hormone-dependent cAMP production. This strategy appeared particularly attractive since hormone stimulation of the V1a

receptor has v[illegible]ually no effect on intracellula[illegible]cAMP levels. Functional analysis of a large number of mutant receptors transiently expressed in COS-7 cells indicated that the presence of V2 receptor sequence at the N terminus of the third **intracellular loop** is critical for efficient activation of Gs. More detailed mutational analysis of this receptor region showed that two polar V2 receptor residues, Gln225 and Glu231, play key roles in Gs recognition. In addition, a short sequence at the N terminus of the cytoplasmic tail was found to make an important contribution to V2 receptor/Gs coupling selectivity. We also made the novel observation that the efficiency of V2 receptor/Gs coupling can be modulated by the length of the central portion of the third **intracellular loop** (rather than the specific amino acid sequence within this domain). These findings provide novel insights into the molecular mechanisms regulating peptide receptor/**G protein** coupling selectivity.

L8 ANSWER 2 OF 79 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 1998395146 MEDLINE
DOCUMENT NUMBER: 98395146 PubMed ID: 9727043
TITLE: Targeting of **G protein**-coupled receptors to the basolateral surface of polarized renal epithelial cells involves multiple, non-contiguous structural signals.
AUTHOR: Saunders C; Keefer J R; Bonner C A; Limbird L E
CORPORATE SOURCE: Department of Pharmacology, Vanderbilt University Medical Center, Nashville, Tennessee 37232-6600, USA.
CONTRACT NUMBER: CA68485 (NCI)
DK 43879 (NIDDK)
TG HL07323 (NHLBI)
+
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1998 Sep 11)** 273 (37) 24196-206.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199810
ENTRY DATE: Entered STN: 19981021
Last Updated on STN: 20000303
Entered Medline: 19981013

AB Truncations and **chimeras** of the alpha2A-adrenergic receptor (alpha2AAR) were evaluated to identify membrane domains responsible for its direct basolateral targeting in Madin-Darby canine kidney cells. An alpha2AAR truncation, encoding transmembrane (TM) regions 1-5, was first delivered basolaterally, but within minutes appeared apically, and at steady-state was primarily lateral in its immunocytochemical localization.
A TM 1-5 truncation with the third **intracellular loop** revealed more intense lateral localization than for the TM 1-5 structure, consistent with the role of the third **intracellular loop** in alpha2AAR stabilization. Addition of TM 6-7 of A1 adenosine receptor (A1AdoR) to alpha2AARTM1-5 creates a **chimera**, alpha2AARTM1-5/A1AdoRTM6-7, which was first delivered apically, resulting either from loss of alpha2AAR sorting information in TM 6-7 or acquisition of apical trafficking signals within A1AdoRTM6-7. Evidence that alpha2AARTM6-7 imparts basolateral targeting information is revealed by the significant basolateral localization of the A1AdoRTM1-5/alpha2AARTM6-7 and A1AdoRTM1-5/alpha2AARTM6-7+i3 **chimeras**, in contrast to the dominant apical localization of A1AdoR. These results reveal that sequences within TM 1-5 and within TM 6-7 of the alpha2AAR confer basolateral targeting, providing the first evidence that alpha2AAR basolateral localization is not conferred by a single region but by non-contiguous membrane-embedded or proximal sequences.

L8 ANSWER 3 OF 79 MEDLINE DUPLICATE 2

ACCESSION NUMBER: 8316326 MEDLINE
DOCUMENT NUMBER: 98316326 PubMed ID: 9651354
TITLE: Differential interactions of the C terminus and the cytoplasmic I-II loop of neuronal Ca2+ channels with **G-protein** alpha and beta gamma subunits. II. Evidence for direct binding.
AUTHOR: Furukawa T; Miura R; Mori Y; Strobeck M; Suzuki K; Ogihara Y; Asano T; Morishita R; Hashii M; Higashida H; Yoshii M; Nukada T
CORPORATE SOURCE: Department of Neurochemistry, Tokyo Institute of Psychiatry, 2-1-8 Kamikitazawa, Setagaya-ku, Tokyo 156, Japan.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1998 Jul 10)** 273 (28) 17595-603.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199808
ENTRY DATE: Entered STN: 19980817
Last Updated on STN: 20000303
Entered Medline: 19980806

AB The present study was designed to obtain evidence for direct interactions of **G-protein** alpha (Galpha) and beta gamma subunits (Gbeta gamma) with N- (alpha1B) and P/Q-type (alpha1A) Ca2+ channels, using synthetic peptides and **fusion** proteins derived from loop 1 (**cytoplasmic loop** between repeat I and II) and the C terminus of these channels. For N-type, prepulse facilitation as mediated by Gbeta gamma was impaired when a synthetic loop 1 peptide was applied intracellularly. Receptor agonist-induced inhibition of N-type as mediated by Galpha was also impaired by the loop 1 peptide but only when applied in combination with a C-terminal peptide. For P/Q-type channels, by contrast, the Galpha-mediated inhibition was diminished by application of a C-terminal peptide alone. Moreover, in vitro binding analysis for N- and P/Q-type channels revealed direct interaction of Galpha with C-terminal **fusion** proteins as well as direct interaction of Gbeta gamma with loop 1 **fusion** proteins. These findings define loop 1 of N- and P/Q-type Ca2+ channels as an interaction site for Gbeta gamma and the C termini for Galpha.

L8 ANSWER 4 OF 79 MEDLINE
ACCESSION NUMBER: 1999060050 MEDLINE
DOCUMENT NUMBER: 99060050 PubMed ID: 9843378
TITLE: SH3 binding domains in the dopamine D4 receptor.
AUTHOR: Oldenhof J; Vickery R; Anafi M; Oak J; Ray A; Schoots O; Pawson T; von Zastrow M; Van Tol H H
CORPORATE SOURCE: Department of Pharmacology, Institute of Medical Science, University of Toronto, Ontario, Canada.
SOURCE: BIOCHEMISTRY, **(1998 Nov 10)** 37 (45) 15726-36.
Journal code: A0G; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199812
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 20000303
Entered Medline: 19981221

AB The dopamine D4 receptor is a **G protein**-coupled receptor (GPCR) that belongs to the dopamine D2-like receptor family. Functionally, the D2-like receptors are characterized by their ability to inhibit adenylyl cyclase. The dopamine D4 receptor as well as many other catecholaminergic receptors contain several putative SH3 binding domains. Most of these sites in the D4 receptor are located in a polymorphic repeat

sequence and flanking sequences in the third **intracellular loop**. Here we demonstrate that this region of the D4 receptor can interact with a large variety of SH3 domains of different origin. The strongest interactions were seen with the SH2-SH3 adapter proteins Grb2 and Nck. The repeat sequence itself is not essential in this interaction. The data presented indicate that the different SH3 domains in the adapter proteins interact in a cooperative fashion with two distinct sites immediately upstream and downstream from the repeat sequence. Removal of all the putative SH3 binding domains in the third **intracellular loop** of the dopamine D4 receptor resulted in a receptor that could still bind spiperone and dopamine. Dopamine could not modulate the coupling of these mutant receptors to adenylyl cyclase and MAPK, although dopamine modulated receptor-**G protein** interaction appeared normal. The receptor deletion mutants show strong constitutive internalization that may account for the deficiency in functional activation of second messengers. The data indicates that the D4 receptor contains SH3 binding sites and that these sites fall within a region involved in the control of receptor internalization.

L8 ANSWER 5 OF 79 MEDLINE
ACCESSION NUMBER: 1999060049 MEDLINE
DOCUMENT NUMBER: 99060049 PubMed ID: 9843377
TITLE: Agonist-mediated downregulation of G alpha i via the alpha 2-adrenergic receptor is targeted by receptor-Gi interaction and is independent of receptor signaling and regulation.
AUTHOR: Jewell-Motz E A; Donnelly E T; Eason M G; Liggett S B
CORPORATE SOURCE: Department of Medicine, University of Cincinnati College of Medicine, Ohio 45267-0564, USA.
CONTRACT NUMBER: HL41496 (NHLBI)
HL53436 (NHLBI)
SOURCE: BIOCHEMISTRY, **(1998 Nov 10)** 37 (45) 15720-5.
Journal code: A0G; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199812
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 20000303
Entered Medline: 19981221

AB One mechanism of long-term agonist-promoted desensitization of alpha2AR function is downregulation of the cellular levels of the alpha subunit of the inhibitory **G protein**, Gi. In transfected CHO cells expressing the human alpha2AAR, a 40.1 +/- 3.3% downregulation of Galphai2 protein occurred after 24 h of exposure of the cells to epinephrine, which was not accompanied by a decrease in Galphai2 mRNA. The essential step that targets Gi for degradation by agonist occupancy of the receptor was explored using mutated alpha2AAR lacking specific structural or functional elements. These consisted of 5HT1A receptor and beta2AR sequences substituted at residues 113-149 of the second **intracellular loop** and 218-235 and 355-371 of the N- and C-terminal regions of the third **intracellular loop** (altered Gi and Gs coupling), deletion of Ser296-299 (absent GRK phosphorylation), and substitution of Cys442 (absent palmitoylation and receptor downregulation). Of these mutants, only those with diminished Gi coupling displayed a loss of agonist-promoted Gi downregulation, thus excluding Gs coupling and receptor downregulation, palmitoylation, and phosphorylation as necessary events. Furthermore, coupling-impaired receptors consisting of mutations in the second or third loops ablated Gi downregulation, suggesting that a discreet structural motif of the receptor is unlikely to represent a key element in the process. While pertussis toxin ablated Gi downregulation, blocking downstream intracellular consequences of alpha2AAR activation or mimicking these pathways by heterologous means

failed to impl e cAMP/adenylyl cyclase, phosp[redacted]ipase C, phospholipase D, or MAP kinase pathways in alpha2AAR-mediated Gi downregulation. Taken together, agonist-promoted Gi downregulation requires physical alpha2AAR-Gi interaction which targets Gi for degradation in a manner that is independent of alpha2AAR trafficking, regulation, or second messengers.

L8 ANSWER 6 OF 79 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 1999019735 MEDLINE
DOCUMENT NUMBER: 99019735 PubMed ID: 9801356
TITLE: **G-protein** beta-subunit specificity in the fast membrane-delimited inhibition of Ca2+ channels.
AUTHOR: Garcia D E; Li B; Garcia-Ferreiro R E; Hernandez-Ochoa E O; Yan K; Gautam N; Catterall W A; Mackie K; Hille B
CORPORATE SOURCE: Department of Physiology and Biophysics, University of Washington, Seattle, Washington 98195, USA.
CONTRACT NUMBER: DA00286 (NIDA)
DA08934 (NIDA)
NS01588 (NINDS)
+
SOURCE: JOURNAL OF NEUROSCIENCE, **(1998 Nov 15)** 18 (22) 9163-70.
Journal code: JDF; 8102140. ISSN: 0270-6474.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199812
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 20000303
Entered Medline: 19981211

AB We investigated which subtypes of **G-protein** beta subunits participate in voltage-dependent modulation of N-type calcium channels. Calcium currents were recorded from cultured rat superior cervical ganglion neurons injected intranuclearly with DNA encoding five different **G-protein** beta subunits. Gbeta1 and Gbeta2 strongly mimicked the fast voltage-dependent inhibition of calcium channels produced by many **G-protein**-coupled receptors. The Gbeta5 subunit produced much weaker effects than Gbeta1 and Gbeta2, whereas Gbeta3 and Gbeta4 were nearly inactive in these electrophysiological studies. The specificity implied by these results was confirmed and extended using the yeast two-hybrid system to test for protein-protein interactions. Here, Gbeta1 or Gbeta2 coupled to the GAL4-activation domain interacted strongly with a channel sequence corresponding to the **intracellular loop** connecting domains I and II of a alpha1 subunit of the class B calcium channel **fused** to the GAL4 DNA-binding domain. In this assay, the Gbeta5 subunit interacted weakly, and Gbeta3 and Gbeta4 failed to interact. Together, these results suggest that Gbeta1 and/or Gbeta2 subunits account for most of the voltage-dependent inhibition of N-type calcium channels and that the linker between domains I and II of the calcium channel alpha1 subunit is a principal receptor for this inhibition.

L8 ANSWER 7 OF 79 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 1998195348 MEDLINE
DOCUMENT NUMBER: 98195348 PubMed ID: 9526000
TITLE: Protein kinase C disrupts cannabinoid actions by phosphorylation of the CB1 cannabinoid receptor.
AUTHOR: Garcia D E; Brown S; Hille B; Mackie K
CORPORATE SOURCE: Department of Physiology and Biophysics, University of Washington, Seattle, Washington 98195, USA.
CONTRACT NUMBER: DA00286 (NIDA)
DA08934 (NIDA)
NS01588 (NINDS)

SOURCE: JOURNAL OF NEUROSCIENCE, **(1998 Apr 15)** 18 (8) 2834-41.
Journal code: JDF; 8102140. ISSN: 0270-6474.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199804
ENTRY DATE: Entered STN: 19980430
Last Updated on STN: 20000303
Entered Medline: 19980420

AB We have found that phosphorylation of a **G-protein**-coupled receptor by protein kinase C (PKC) disrupts modulation of ion channels by the receptor. In AtT-20 cells transfected with rat cannabinoid receptor (CB1), the activation of an inwardly rectifying potassium current (Kir current) and depression of P/Q-type calcium channels by cannabinoids were prevented by stimulation of protein kinase C by 100 nM phorbol 12-myristate 13-acetate (PMA). In contrast, activation of Kir current by somatostatin was unaffected, and inhibition of calcium channels was only modestly attenuated. The possibility that PKC acted by phosphorylating CB1 receptors was confirmed by demonstrating that PKC phosphorylated a single serine (S317) of a **fusion** protein incorporating the third **intracellular loop** of CB1. Mutating this serine to alanine did not affect the ability of CB1 to modulate currents, but it eliminated disruption by PMA, demonstrating that PKC can disrupt ion channel modulation by receptor phosphorylation.

L8 ANSWER 8 OF 79 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1999:24754 BIOSIS
DOCUMENT NUMBER: PREV199900024754
TITLE: Dopamine D1B receptor **chimeras** reveal modulation of partial agonist activity by carboxyl-terminal tail sequences.
AUTHOR(S): Sugamori, Kim S.; Scheideler, Mark A.; Vernier, Philippe; Niznik, Hyman B. (1)
CORPORATE SOURCE: (1) Lab. Mol. Neurobiol., Centre Addiction Mental Health, 250 College St., Toronto, ON M5T 1R8 Canada
SOURCE: Journal of Neurochemistry, (**Dec., 1998**) Vol. 71, No. 6, pp. 2593-2599.
ISSN: 0022-3042.
DOCUMENT TYPE: Article
LANGUAGE: English

AB NNC 01-0012, a second-generation benzazepine compound, pharmacologically differentiates multiple vertebrate D1 receptor subtypes (D1A, D1B, D1C, and D1D) and displays high selectivity and affinity for dopamine D1C receptors. Functionally, whereas NNC 01-0012 acts as a full or poor antagonist at D1C and D1A receptormediated cyclic AMP production, respectively, it exhibits partial agonist activity at the D1B receptor. To define some of the structural motifs that regulate the pharmacological and functional differentiation of vertebrate dopamine D1 receptors by NNC 01-0012, a series of receptor **chimeras** were constructed in which the divergent carboxyl-terminal (CT) receptor tails were replaced with the corresponding sequences of D1A, D1B, or D1C receptors. Substitution of the vertebrate D1B carboxyl-terminal-tail at position Tyr345 with carboxyl-terminal-tail sequences of the D1A receptor abolished the partial agonist activity of NNC 01-0012 without affecting dopamine stimulated cyclic AMP accumulation. At vertebrate D1B/D1CcT-tail receptor mutants, however, the intrinsic activity of the partial agonist NNC 01-0012 (10 muM) was markedly enhanced (apprx60% relative to 10 muM dopamine) with no concomitant alteration in the molecule's ligand binding affinity or

constitutive activity of the **chimeric** receptor. Similar results were obtained with other benzazepines such as SKF-38393 and SCH-23390, which act as partial agonists at vertebrate D1B receptors. Substitution of D1A and D1C receptor carboxyl-terminal tails with sequences encoded by the D1B receptor carboxyl-terminal tail did not, however, produce receptors with functional characteristics significantly different from wild type. Taken together, these data clearly suggest that in addition to well-characterized domains and amino acid residues in the third **cytoplasmic loop**, partial agonist activity at the D1B receptor is modulated by sequence-specific motifs within the carboxyl-terminal tail, a region that may underlie the possible structural basis for functionally divergent roles of multiple dopamine D1-like receptors.

L8 ANSWER 9 OF 79 MEDLINE
ACCESSION NUMBER: 1998190081 MEDLINE
DOCUMENT NUMBER: 98190081 PubMed ID: 9524122
TITLE: GPR1 encodes a putative **G protein** -coupled receptor that associates with the Gpa2p Galpha subunit and functions in a Ras-independent pathway.
AUTHOR: Xue Y; Batlle M; Hirsch J P
CORPORATE SOURCE: Department of Cell Biology and Anatomy, Mount Sinai School of Medicine, New York, NY 10029, USA.
SOURCE: EMBO JOURNAL, **(1998 Apr 1)** 17 (7) 1996-2007. Journal code: EMB; 8208664. ISSN: 0261-4189.
PUB. COUNTRY: ENGLAND: United Kingdom Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-Z74083
ENTRY MONTH: 199806
ENTRY DATE: Entered STN: 19980611 Last Updated on STN: 20000303 Entered Medline: 19980601

AB The yeast RAS1 and RAS2 genes appear to be involved in control of cell growth in response to nutrients. Here we show that this growth control also involves a signal mediated by the heterotrimeric **G protein** alpha subunit homolog encoded by GPA2. A GPA2 null allele conferred a severe growth defect on cells containing a null allele of RAS2, although either mutation alone had little effect on growth rate. A constitutive allele of GPA2 could stimulate growth of a strain lacking both RAS genes. Constitutive GPA2 conferred heat shock sensitivity on both wild-type cells and cells lacking RAS function, but had no effect in a strain containing a null allele of SCH9, which encodes a kinase related to protein kinase A. The GPR1 gene was isolated and was found to encode a protein with the characteristics of a **G protein** -coupled receptor. Double Deltagpr1 Deltaras2 mutants displayed a severe growth defect that was suppressed by expression of the constitutive allele of GPA2, confirming that GPR1 acts upstream of GPA2. Gpr1p is expressed on the cell surface and requires sequences in the membrane-proximal region of its third **cytoplasmic loop** for function, as expected for a **G protein**-coupled receptor. GPR1 RNA was induced when cells were starved for nitrogen and amino acids. These results are consistent with a model in which the GPR1/GPA2 pathway activates the Sch9p kinase to generate a response that acts in parallel with that generated by the Ras/cAMP pathway, resulting in the integration of nutrient signals.

L8 ANSWER 10 OF 79 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 1998266974 MEDLINE

DOCUMENT NUMBER: 66974 PubMed ID: 9605937
TITLE: Identification of binding domains of the growth hormone-releasing hormone receptor by analysis of mutant and **chimeric** receptor proteins.
AUTHOR: DeAlmeida V I; Mayo K E
CORPORATE SOURCE: Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, Illinois 60208, USA.
CONTRACT NUMBER: DK-48071 (NIDDK)
SOURCE: MOLECULAR ENDOCRINOLOGY, **(1998 May)** 12 (5) 750-65.
Journal code: NGZ; 8801431. ISSN: 0888-8809.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199809
ENTRY DATE: Entered STN: 19981006
Last Updated on STN: 19981006
Entered Medline: 19980923

AB The hypothalamic peptide GH-releasing hormone (GHRH) stimulates the release of GH from the pituitary through binding and activation of the GHRH receptor, which belongs to the family of **G protein** -coupled receptors. The objective of this study was to identify regions of the receptor critical for interaction with the ligand by expressing and analyzing truncated and **chimeric** epitope-tagged GHRH receptors. Two truncated receptors, GHRHdeltaN, in which part of the N-terminal domain between the putative signal sequence and the first transmembrane domain was deleted, and GHRHdeltaC, which was truncated downstream of the first **intracellular loop**, were generated. Both the receptors were deficient in ligand binding, indicating that neither the N-terminal extracellular domain (N terminus) nor the membrane-spanning domains with the associated extracellular loops (C terminus) are alone sufficient for interaction with GHRH. In subsequent studies, **chimeric** proteins between the receptors for GHRH and vasoactive intestinal peptide (VIP) or secretin were generated, using the predicted start of the first transmembrane domain as the junction for the exchange of the N terminus between receptors. The **chimeras** having the N terminus of the GHRH receptor and the C terminus of either the VIP or secretin receptor (GNVC and GNSC) did not bind GHRH or activate adenylate cyclase after GHRH treatment. The reciprocal **chimeras** having the N terminus of either the VIP or secretin receptors and the C terminus of the GHRH receptor (VNGC and SNGC) bound GHRH and stimulated cAMP accumulation after GHRH treatment. These results suggest that although the N-terminal extracellular domain is essential for ligand binding, the transmembrane domains and associated extracellular loop regions of the GHRH receptor provide critical information necessary for specific interaction with GHRH.

L8 ANSWER 11 OF 79 BIOTECHNO COPYRIGHT 2001 Elsevier Science B.V.
ACCESSION NUMBER: 1998:29022184 BIOTECHNO
TITLE: Purification of G.alpha.(i,2) subunit from bovine brain by affinity chromatography
AUTHOR: Simonovic M.; Soskic V.; Joksimovic J.
CORPORATE SOURCE: Dr. V. Soskic, Institute of Biological Research, 29. Novembra 142, 11060 Belgrade, Yugoslavia.
E-mail: vsoskic@ibiss.bg.ac.yu
SOURCE: Jugoslovenska Medicinska Biokemija, **(1998)**, 17/4 (385-389), 22 reference(s)
CODEN: JMBIEE ISSN: 0354-3447
DOCUMENT TYPE: Journal; Article
COUNTRY: Yugoslavia
LANGUAGE: English
SUMMARY LANGUAGE: English; Serbo-Croatian

AN 1998:29022184 BIOTECHNO
AB In order to purify G.alpha.-proteins from bovine brain, novel affinity

chromatography medium was prepared and the method developed described in detail. For this purpose the third **intracellular loop** of the human dopamine receptor D(2s)-CL3 was cloned and expressed in E. coli BL21 DE3 as a **fusion** protein with glutathione-S-transferase (D(2s)-CL3-GST) and pGEX-2T serving as the expression vector.

The resulting construct was purified by Glutathione- Sepharose affinity chromatography using Tris-buffered glutathione solution as the eluent. Synaptosomal membranes of the bovine audate nuclei were used as a source of G-proteins. **G-protein**-enriched fraction isolated by DEAE-Sephacel ion-exchange chromatography was further purified on the affinity column of the D(2s)-CL3-GST protein immobilized on Glutathione-Sepharose. Bound protein was eluted with 10 mmol/L glutathione solution and the fractions were subjected to SDS-polyacrylamide slab gel electrophoresis and Western blot analysis employing polyclonal antiserum P-960 raised against G.alpha.(i,2)-subunit. These analyses demonstrated a high purity degree of the G.alpha.(i,2)- protein upon affinity chromatography on Glutathione-Sepharose/D(2s)-CL3-GST column.

L8 ANSWER 12 OF 79 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 1999150886 MEDLINE
DOCUMENT NUMBER: 99150886 PubMed ID: 10026824
TITLE: Molecular aspects of vasopressin receptor function.
AUTHOR: Schoneberg T; Kostenis E; Liu J; Gudermann T; Wess J
CORPORATE SOURCE: Laboratory of Bioorganic Chemistry, NIH-NIDDK, Bethesda, Maryland 20892, USA.
SOURCE: ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY, **(1998)** 449 347-58.
Journal code: 2LU; 0121103. ISSN: 0065-2598.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY DATE: Entered STN: 19990324
Last Updated on STN: 20000303
Entered Medline: 19990310

AB The molecular mechanisms governing the **G protein** coupling selectivity of different members of the vasopressin receptor family were studied by using a combined molecular genetic/biochemical approach. While the V1a and V1b vasopressin receptors are selectively linked to G proteins of the Gq/11 class, the V2 vasopressin receptor is preferentially coupled to Gs. Systematic functional analysis of V1a/V2 hybrid receptors showed that the second **intracellular loop** of the V1a receptor is required and sufficient for efficient coupling to Gq/11, whereas the third **intracellular loop** of the V2 receptor is required and sufficient for coupling to Gs. By using a strategy involving the coexpression of the wild type V1a receptor with **chimeric G protein** alpha s/alpha q subunits, two C-terminal alpha q/11 residues were identified that are critical for proper receptor recognition. We previously demonstrated -in transiently transfected COS-7 cells- that selected mutant V2 vasopressin receptors (all of which have been identified in X-linked nephrogenic diabetes insipidus patients) containing inactivating mutations in the C-terminal third of the receptor protein (including missense, frameshift, or nonsense mutations) can be functionally rescued by coexpression with a C-terminal V2 receptor fragment (V2-tail) spanning the region where the various mutations occur. Co-immunoprecipitation experiments and a newly developed sandwich ELISA revealed that the V2-tail polypeptide directly interacts with the mutant V2 receptors thus creating a functional receptor protein. To study the potential therapeutic usefulness of these findings, CHO cell lines stably expressing low levels of functionally inactive mutant V2 vasopressin receptors (E242stop, Y280C, and W284stop) were created and infected with a recombinant adenovirus coding for the V2-tail polypeptide.

Following adenovirus infection, arginine vasopressin (AVP) gained the

ability to stim[redacted]te cAMP formation in all CHO c[redacted] clones studied. Adenovirus-mediated gene transfer also proved to be a highly efficient method to achieve expression of the V2-tail fragment (as well as of the wild type V2 vasopressin receptor) in MDCK renal tubular cells. We therefore speculate that the targeted expression of receptor fragments in vivo may represent a novel strategy in the treatment of human diseases caused by inactivating mutations in distinct **G protein** -coupled receptors.

L8 ANSWER 13 OF 79 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 1998430723 MEDLINE
DOCUMENT NUMBER: 98430723 PubMed ID: 9759923
TITLE: Quantification of human dopamine D2s receptor interactions with G alpha(i,1,2)- and G alpha(o)-proteins.
AUTHOR: Simonovic M; Soskic V; Joksimovic J
CORPORATE SOURCE: Institute of Chemistry, Technology and Metallurgy, Belgrade, Yugoslavia.
SOURCE: NEUROCHEMISTRY INTERNATIONAL, **(1998 Sep)** 33 (3) 271-5.
Journal code: BNU; 8006959. ISSN: 0197-0186.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199812
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 20000303
Entered Medline: 19981211

AB A simple and rapid in vitro method for qualitative and quantitative estimation of the G alpha-subunits interaction with the third **intracellular loop** of human D2s dopamine receptor has been developed. For this purpose, D2s-CL3 was cloned in pGEX-2T vector and expressed in E. coli BL21 DE3 as a **fusion** protein with glutathione-S-transferase (D2s-CL3-GST). The resulting soluble construct was purified by affinity chromatography on glutathione-Sepharose. G alpha-subunits were expressed and purified as His-tagged proteins. For the assay of G alpha/D2s-CL3-GST interactions, varying concentrations of pure His-tagged G alpha-proteins were immobilized on His-Bind Resin and titrated with D2s-CL3-GST **fusion** protein. G alpha/D2s-CL3-GST interactions were quantified by GST activity determination assay. It was shown that the **fusion** protein interacts specifically with different G alpha proteins, especially with G alpha(i) proteins. Based on saturation binding analyses, Kd values were determined revealing the highest affinity of His-G alpha(i,2) binding to the **fusion** protein. The affinities for G alpha(i)/D2s-CL3-GST protein interactions estimated in this way were in nanomolar range of concentrations.

L8 ANSWER 14 OF 79 BIOTECHNO COPYRIGHT 2001 Elsevier Science B.V.
ACCESSION NUMBER: 1998:29034235 BIOTECHNO
TITLE: Role of the third **intracellular loop** of the alpha-2 adrenergic receptor in regulating receptor density
AUTHOR: Heck D.A.; Bylund D.B.
CORPORATE SOURCE: Dr. D.B. Bylund, Department of Pharmacology, University Nebraska Medical Center, 600 South 42(nd) Street, Omaha, NE 68198-6260, United States.
E-mail: dbylund@mail.unmc.edu
SOURCE: Pharmacology Reviews and Communications, (**1998**), 10/2 (101-110), 23 reference(s)
CODEN: PHRCF6 ISSN: 1028-8945
DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1998:29034235 BIOTECHNO
AB Our laboratory has previously shown that the mechanism of down-regulation

of alpha-2 adrgic receptor subtypes is an increase in the rate constant for receptor disappearance. In addition, we have found subtype-specific differences in the regulation of receptor density in the presence of norepinephrine. For example, blocking functional **G protein** coupling with pertussis toxin alters the time-course of norepinephrine-induced down-regulation for alpha-2A receptors while having little effect on the time-course of receptor down-regulation for alpha-2B receptors. In contrast, treatment with pertussis toxin alone decreases alpha-2B receptor density while having little effect on alpha-2A receptor density. To explore these subtype-specific differences, we constructed a **chimeric** receptor in which the third **intracellular loop** of the alpha-2B receptor was replaced with the third **intracellular loop** of the alpha-2A receptor. We found that the **chimeric** receptor exhibits similar characteristics to the wild-type receptor in terms of radioligand binding, potency of norepinephrine to down-regulate receptor density, and effects of pertussis toxin on receptor density. In contrast, we found that replacement of the third **intracellular loop** of the alpha-2B receptor with that of the alpha-2A receptor alters the regulation of receptor density in both the absence and presence of norepinephrine.

L8 ANSWER 15 OF 79 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 97467344 MEDLINE
DOCUMENT NUMBER: 97467344 PubMed ID: 9325274
TITLE: The C-terminal third **intracellular loop** of the rat AT1A angiotensin receptor plays a key role in **G protein** coupling specificity and transduction of the mitogenic signal.
AUTHOR: Conchon S; Barrault M B; Miserey S; Corvol P; Clauser E
CORPORATE SOURCE: INSERM Unite 36, College de France, 3, rue d'Ulm 75005 Paris, France.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1997 Oct 10)** 272 (41) 25566-72.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199711
ENTRY DATE: Entered STN: 19971224
Last Updated on STN: 20000303
Entered Medline: 19971113

AB To identify the role(s) of the third **intracellular loop** of the angiotensin II (AngII) type 1A (AT1A) receptor in **G protein** coupling specificity and receptor activation, several **chimerae** were constructed and characterized. The cDNA sequence encoding the C-terminal segment of the third **intracellular loop** of the AT1A receptor (residues 234-240) was replaced with the homologous regions of the alpha1B adrenergic (alpha1B-AR), the beta2 adrenergic (beta2-AR), and the AngII type 2 (AT2) receptors. These **chimeric** receptors were stably expressed in Chinese hamster ovary cells, and their pharmacological and functional properties were characterized, including AngII-induced inositol phosphate and cyclic AMP (cAMP) productions, [3H]thymidine incorporation into DNA, and internalization. The affinities of these **chimeric** receptors for [Sar1]AngII, [Sar1,Ile8]AngII, and losartan were essentially normal; however, the affinity of these mutants was increased by a factor of 10-40 for the AT2-specific ligand CGP42112A. The functional properties of the alpha1B-AR **chimera** were essentially identical to those of the wild type AT1A receptor. On the other hand, replacement with the beta2-AR segment produced a partial reduction of the inositol phosphate production, a measurable AngII-induced cAMP accumulation, a reduced internalization, and a total impairment to transduce the mitogenic effect of AngII. The AT2

chimera present[redacted]a normal internalization, but [redacted] inactive in all the other functional tests. In conclusion, the distal segment of the third **intracellular loop** of the rat AT1A receptor plays a pivotal role in coupling selectivity and receptor signaling via **G protein**(s) as well as in the activation of the specific signaling pathways involved in the mitogenic actions of AngII.

L8 ANSWER 16 OF 79 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 97400575 MEDLINE
DOCUMENT NUMBER: 97400575 PubMed ID: 9252410
TITLE: Stimulus-dependent phosphorylation of **G-protein**-coupled receptors by casein kinase 1alpha.
AUTHOR: Tobin A B; Totty N F; Sterlin A E; Nahorski S R
CORPORATE SOURCE: Department of Cell Physiology and Pharmacology, University of Leicester, University Road, Leicester LE1 9HN, United Kingdom.. TBA@le.ac.uk
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1997 Aug 15)** 272 (33) 20844-9.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199709
ENTRY DATE: Entered STN: 19970916
Last Updated on STN: 20000303
Entered Medline: 19970904

AB We have previously demonstrated that the phospholipase C-coupled m3-muscarinic receptor is phosphorylated in an agonist-sensitive manner by a protein kinase of approximately 40 kDa purified from porcine cerebellum (Tobin, A. B., Keys, B., and Nahorski, S. R. (1996) J. Biol Chem. 271, 3907-3916). This kinase, called muscarinic receptor kinase (MRK), is distinct from second messenger-regulated protein kinases and from beta-adrenergic receptor kinase and other members of the **G-protein**-coupled receptor kinase family. In the present study we propose that MRK is casein kinase 1alpha (CK1alpha) based on the following evidence: 1) the amino acid sequence from two proteolytic peptide fragments derived from purified MRK corresponded exactly to sequences within CK1alpha. 2) Casein kinase activity co-eluted with MRK activity from the final two chromatography steps in the purification of porcine brain MRK. 3) Recombinant CK1alpha expressed in Sf9 cells is able to phosphorylate both casein and the bacterial **fusion** protein, Ex-m3, that contains a portion of the third **intracellular loop** of the m3-muscarinic receptor downstream of glutathione S-transferase. 4) Partially purified CK1alpha increased the level of muscarinic receptor phosphorylation in an agonist-sensitive manner when reconstituted with membranes from Chinese hamster ovary-m3 cells expressing the human recombinant m3-muscarinic receptor. 5) Partially-purified CK1alpha phosphorylated rhodopsin, contained in urea-treated bovine rod outer segment membranes, and the extent of phosphorylation was increased in the presence of light. These data demonstrate that the kinase previously called MRK is CK1alpha, and that CK1alpha offers an alternative protein kinase pathway from that of the **G-protein**-coupled receptor kinase family for the stimulus-dependent phosphorylation of the m3-muscarinic receptor, rhodopsin, and possibly other **G-protein**-coupled receptors.

L8 ANSWER 17 OF 79 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 97390392 MEDLINE
DOCUMENT NUMBER: 97390392 PubMed ID: 9242625
TITLE: Identification of membrane insertion sequences of the rabbit gastric cholecystokinin-A receptor by in vitro translation.
AUTHOR: Bayle D; Weeks D; Sachs G
CORPORATE SOURCE: UCLA and the Wadsworth Veterans Administration Hospital, Los Angeles, California 90073, USA.

CONTRACT NUMBER: 294 (NIDDK)
DK40615 (NIDDK)
DK41301 (NIDDK)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1997 Aug 8)** 272 (32) 19697-707.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U88601; GENBANK-X64694
ENTRY MONTH: 199709
ENTRY DATE: Entered STN: 19970922
Last Updated on STN: 19970922
Entered Medline: 19970905

AB To determine which amino acid sequences account for transmembrane folding of G7 receptors, the membrane domain of the rabbit cholecystokinin-A (CCK-A) **G-protein**-coupled receptor has been investigated by in vitro transcription/translation of two types of **fusion** vectors containing sequences that include putative transmembrane segments. First, the sèven putative transmembrane domains of the CCK-A receptor were inserted individually into pGEM vectors beginning with the cDNA encoding the first 101 (HK-M0) or 139 (HK-M1) amino acids of the alpha subunit of the gastric H, K-ATPase. These were separated by the cDNA for the inserted transmembrane domains from the cDNA encoding the last 177 amino acids of the beta subunit of the H,K-ATPase containing five N-linked glycosylation consensus sequences (Bamberg, K., and Sachs, G. (1994) J. Biol. Chem. 269, 16909-16919). Transcription/translation of these **fusion** vectors in rabbit reticulocyte lysate +/- dog pancreatic microsomes followed by SDS-polyacrylamide gel electrophoresis defined the presence of signal anchor sequences in HK-M0 by glycosylation and stop transfer sequences in HK-M1 by inhibition of glycosylation. Six out of the seven putative transmembrane domains had membrane insertion signals, but no membrane insertion activity was found for the H3 segment in these vectors. To test the effect of specific upstream and downstream sequences on membrane insertion, vectors were also made starting with the cDNA encoding the N terminus of the CCK-A receptor separated from the last 177 amino acids of the H,K-ATPase beta subunit by cDNA encoding CCK-A receptor sequences of different lengths. In addition to transcription/translation, endoglycosidase H treatment was used to verify glycosylation when multiple bands were found in the presence of microsomes. The four positive charges in the loop between H1 and H2 were required for the correct orientation of the first transmembrane domain. The H3 segment acted as a stop transfer sequence only when the whole N terminus and H3 were followed by the positive charges in the **cytoplasmic loop** between H3 and H4. The activity of H6 as a signal anchor sequence depended on preceding positive charges. These translation data using two types of **fusion** vectors establish a **seven-transmembrane** folding model using only in vitro translation for the CCK-A receptor beginning with two signal anchor sequences and then alternating stop transfer and signal anchor insertions.
Positive charges between H1 and H2, H3 and H4, and H5 and H6 function as cytoplasmic anchors in the membrane folding of this receptor.

L8 ANSWER 18 OF 79 MEDLINE DUPLICATE 11
ACCESSION NUMBER: 97362282 MEDLINE
DOCUMENT NUMBER: 97362282 PubMed ID: 9211939
TITLE: Interaction of arrestins with intracellular domains of muscarinic and alpha2-adrenergic receptors.
AUTHOR: Wu G; Krupnick J G; Benovic J L; Lanier S M
CORPORATE SOURCE: Department of Pharmacology, Medical University of South Carolina, Charleston, South Carolina 29425, USA.
CONTRACT NUMBER: GM47419 (NIGMS)
NS24821 (NINDS)

SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (**7 Jul 11**) 272 (28) 17836-42.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199708
ENTRY DATE: Entered STN: 19970825
Last Updated on STN: 19970825
Entered Medline: 19970814

AB The intracellular domains of **G-protein**-coupled receptors provide sites for interaction with key proteins involved in signal initiation and termination. As an initial approach to identify proteins interacting with these receptors and the receptor motifs required for such interactions, we used intracellular subdomains of **G-protein**-coupled receptors as probes to screen brain cytosol proteins. Peptides from the third **intracellular loop** (i3) of the M2-muscarinic receptor (MR) (His208-Arg387), M3-MR (Gly308-Leu497), or alpha2A/D-adrenergic receptor (AR) (Lys224-Phe374) were generated in bacteria as glutathione S-transferase (GST) **fusion** proteins, bound to glutathione-Sepharose and used as affinity matrices to detect interacting proteins in fractionated bovine brain cytosol. Bound proteins were identified by immunoblotting following SDS-polyacrylamide gel electrophoresis. Brain arrestins bound to the GST-M3 **fusion** protein, but not to the control GST peptide or i3 peptides derived from the alpha2A/D-AR and M2-MR. However, each of the receptor subdomains bound purified beta-arrestin and arrestin-3. The interaction of the M3-MR and M2-MR i3 peptides with arrestins was further investigated. The M3-MR i3 peptide bound in vitro translated [3H]beta-arrestin and [3H]arrestin-3, but did not interact with in vitro translated or purified visual arrestin. The properties and specificity of the interaction of in vitro translated [3H]beta-arrestin, [3H]visual arrestin, and [3H]beta-arrestin/visual arrestin **chimeras** with the M2-MR i3 peptide were similar to those observed with the intact purified M2-MR that was phosphorylated and/or activated by agonist. Subsequent binding site localization studies indicated that the interaction of beta-arrestin with the M3-MR peptide required both the amino (Gly308-Leu368) and carboxyl portions (Lys425-Leu497) of the receptor subdomain. In contrast, the carboxyl region of the M3-MR i3 peptide was sufficient for its interaction with arrestin-3.

L8 ANSWER 19 OF 79 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 97238831 MEDLINE
DOCUMENT NUMBER: 97238831 PubMed ID: 9083028
TITLE: First **intracellular loop** of the human cholecystokinin-A receptor is essential for cyclic AMP signaling in transfected HEK-293 cells.
AUTHOR: Wu V; Yang M; McRoberts J A; Ren J; Seensalu R; Zeng N; Dagrag M; Birnbaumer M; Walsh J H
CORPORATE SOURCE: CURE/Digestive Diseases Research Center, Division of Digestive Diseases, Department of Medicine, UCLA School of Medicine, and West Los Angeles Veterans Administration Medical Center, Los Angeles, California 90073,. USA.vwu@ucla.edu
CONTRACT NUMBER: DK-10054 (NIDDK)
DK-17294 (NIDDK)
DK-40301 (NIDDK)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1997 Apr 4)** 272 (14) 9037-42.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199705
ENTRY DATE: Entered STN: 19970514
Last Updated on STN: 20000303

ered Medline: 19970508

AB Cholecystokinin (CCK)-A and CCK-B receptors are highly homologous members of the **seven transmembrane** domain **G-protein**-coupled receptor superfamily. Genes of both receptors contain five exons and share a similar exon-intron organization. To determine the structural basis of CCK-A receptor (CCK-AR) functionally coupled to Gs, a series of **chimeric** mutants were constructed by replacing exons of human CCK-B receptor (CCK-BR), from the second to the fifth (last) exon, with human CCK-AR counterparts. Binding and signal transduction properties of wild-type and **chimeric** receptors were examined in stably transfected HEK-293 cells. **Chimeric** receptors that maintained high affinity binding to CCK exhibited dose-dependent increases in intracellular calcium mobilization similar to both wild-type receptors. However, only the wild-type CCK-AR and **chimeric** mutants containing the second exon of CCK-AR were able to mediate significantly greater increases in intracellular cAMP content and adenylyl cyclase activity compared with wild-type CCK-BR. A CCK-BR mutant was further constructed by replacing five amino acids, Gly-Leu-Ser-Arg-(Arg)-Leu, in the first **intracellular loop** with the corresponding five CCK-AR specific amino acids, Ile-Arg-Asn-Lys-(Arg)-Met. The resultant receptor maintained high affinity binding to both CCK and gastrin and dose-dependent calcium responses similar to wild-type CCK-BR. However, this first **intracellular loop** mutant also gained positive cAMP responses to both sulfated CCK-8 and gastrin-17 with EC50 values of 8.5 +/- 1 nM and 23 +/- 7 nM, respectively. These data suggest that the first **intracellular loop** of CCK-AR is essential for coupling to Gs and activation of adenylyl cyclase signal transduction cascade.

L8 ANSWER 20 OF 79 MEDLINE DUPLICATE 13

ACCESSION NUMBER: 97365170 MEDLINE
DOCUMENT NUMBER: 97365170 PubMed ID: 9220972
TITLE: Role of the amino terminus of the third **intracellular loop** in agonist-promoted downregulation of the alpha2A-adrenergic receptor.
AUTHOR: Jewell-Motz E A; Donnelly E T; Eason M G; Liggett S B
CORPORATE SOURCE: Departments of Medicine and Molecular Genetics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267, USA.
CONTRACT NUMBER: HL07382 (NHLBI)
HL53436 (NHLBI)
SOURCE: BIOCHEMISTRY, **(1997 Jul 22)** 36 (29) 8858-63.
Journal code: A0G; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199709
ENTRY DATE: Entered STN: 19970922
Last Updated on STN: 20000303
Entered Medline: 19970908

AB A prominent feature of long-term regulation of the alpha2A-adrenergic receptor (alpha2AAR) is a loss of cellular receptors over time (downregulation). The molecular determinants of downregulation were sought by targeting regions of the receptor involved in **G protein** coupling and phosphorylation. Mutated receptors, consisting of **chimeric** substitutions of analogous beta2-adrenergic receptor (beta2AR) and serotonin 5-hydroxytryptamine1A (5-HT1A) receptor sequence into the second **intracellular loop** (ICL2) (residues 113-149), the amino terminus (residues 218-235) and carboxy terminus (residues 355-371) of ICL3, and a deletion of the beta-adrenergic receptor kinase (betaARK) phosphorylation sites in the third **intracellular loop** (ICL3) (residues 293-304), were expressed in Chinese hamster ovary (CHO) cells. Wild-type alpha2AAR underwent 31% +/- 3% downregulation after 24 h of exposure to 100 microM epinephrine. Loss of downregulation was observed with some

mutants, but th as not related to functional c ling to inhibitory or stimulatory guanine nucleotide regulatory binding proteins (Gi or GS) or to phosphorylation. Rather, any mutant with a substitution of the amino terminus of ICL3 (regardless of whether the substitution was with beta2AR or 5-HT1A sequence) resulted in upregulation. Studies with an inhibitor of protein synthesis indicated that the primary mechanism of downregulation of the alpha2AAR is agonist-promoted degradation of receptor protein which requires a destabilization sequence in the amino terminus of ICL3. Thus, in contrast to other **G protein**-coupled receptors, in which **G protein** coupling or phosphorylation are critical for long-term agonist regulation, the alpha2AAR has a specific structural domain distinct from these other functional regions that serves to direct agonist-promoted downregulation.

L8 ANSWER 21 OF 79 MEDLINE DUPLICATE 14
ACCESSION NUMBER: 97207241 MEDLINE
DOCUMENT NUMBER: 97207241 PubMed ID: 9054376
TITLE: The thrombin receptor second **cytoplasmic loop** confers coupling to Gq-like G proteins in **chimeric** receptors. Additional evidence for a common transmembrane signaling and **G protein** coupling mechanism in **G protein**-coupled receptors.
AUTHOR: Verrall S; Ishii M; Chen M; Wang L; Tram T; Coughlin S R
CORPORATE SOURCE: Cardiovascular Research Institute, University of California, San Francisco, California 94143-0130, USA.
CONTRACT NUMBER: HL44907 (NHLBI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1997 Mar 14)** 272 (11) 6898-902.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199704
ENTRY DATE: Entered STN: 19970424
Last Updated on STN: 20000303
Entered Medline: 19970417

AB Thrombin activates human platelets and other cells in part by cleaving an unusual **G protein**-coupled receptor. Thrombin cleavage of this receptor's amino-terminal exodomain unmasks a new amino terminus. This then binds intramolecularly to the body of the receptor to trigger transmembrane signaling and activation of Gi- and Gq-like G proteins. Toward identifying the domains responsible for thrombin receptor-**G protein** interactions, we examined the signaling properties of **chimeric** receptors in which thrombin receptor cytoplasmic sequences replaced the cognate sequences in the Gs-coupled beta2-adrenergic receptor (beta2AR) or the Gi-coupled dopamine D2 receptor (D2R). In Xenopus oocytes, a **chimeric** beta2AR bearing the thrombin receptor second cytoplasmic (C2) loop gained the ability to trigger intracellular Ca2+ release in response to adrenergic agonist, whereas a beta2AR bearing the cognate C2 loop from the D2R did not. Similarly, in COS-7 cells, a **chimeric** D2R bearing the thrombin receptor C2 loop gained the ability to trigger phosphoinositide hydrolysis in response to dopaminergic agonist, apparently by coupling to a Gq-like **G protein**. No detectable Gs coupling was seen. Thus, the thrombin receptor C2 loop was able to confer Gq-like coupling in several different receptor contexts. These observations suggest that the thrombin receptor C2 loop specifies Gq coupling by directly contacting Gq or by contributing to a structure required for Gq coupling. The ability of the thrombin receptor C2 loop to function in the context of the D2R and beta2AR strongly suggests that the transmembrane switching and **G protein** activation strategies used by the thrombin receptor must be very similar to those used by the D2R and beta2AR despite the thrombin